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Plasmodium vivax VK247 and VK210 Circumsporozoite Proteins in *Anopheles* Mosquitoes from Andoas, Peru

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ABSTRACT *Anopheles* mosquitoes captured in Andoas, Peru, a *Plasmodium vivax*-endemic area in the Peruvian Amazon region, contained both VK210 and VK247 *P. vivax* circumsporozoite (CS) proteins. Approximately 0.9% of the 4,403 mosquitoes tested by enzyme-linked immunosorbent assay were positive, 28% and 72% of the positive mosquitoes contained VK210 and VK247 CS proteins, respectively. These findings correlate strongly with a recent report of the presence of antibodies in residents of this area that recognize the VK210 and VK247 repeats, clearly indicating that both *P. vivax* CS protein polymorphs are common in the region.

KEY WORDS Anopheles, *Plasmodium* spp., circumsporozoite

THE RECENT DETECTION of a variant (VK247) form of the *Plasmodium vivax* circumsporozoite (CS) protein has attracted much attention. Before the first report of phenotypic heterogeneity in Thailand (Rosenberg et al. 1989), the *P. vivax* CS protein was generally considered invariant and thus an excellent candidate antigen for vaccine trials. Other reports have presented evidence of anti-VK247 human antibody in sera from Brazil and India (Cochrane et al. 1990) and Thailand (Wirtz et al. 1990). The global distribution of the VK247 CS gene has also been described providing evidence of its presence in South America, West Africa, and the Indian subcontinent (Kain et al. 1991). Such information indicates that a single-epitope vaccine based on the VK210 form alone will be ineffective (Kain et al. 1991) and may in fact select for variant forms (Wirtz et al. 1990).

Recently, the prevalence of human antibody to the VK247 repeat of the *P. vivax* CS protein was reported from Andoas, a malaria endemic area in northern Peru (Franke et al. 1992). In that study, a significant proportion (60%) of the *P. vivax*-positive individuals was found to have antibodies to the *P. vivax* VK247 CS protein.

An *Anopheles* vector study was begun in that site in 1990 using the VK210 CS protein enzyme-linked immunosorbent assay (ELISA). After the discovery of the antibody response of the indigenous population to the VK247 repeat region, it was decided to retest those same captured mosquitoes to determine the presence and prevalence of the VK247 CS protein. Those findings are discussed here.

Materials and Methods

Mosquito Collections. Andoas is located 350 km WNW of Iquitos on the Pastaza River, near the Ecuadorian border. Several small villages totaling ≈500 indigenous persons have developed around an oil exploration camp set up by the Occidental Petroleum Corporation of Peru in 1973. The typical vegetation surrounding the villages is tropical rain forest, the mean annual temperature is ≈25.1°C, and the annual rainfall exceeds 250 cm (Peñaherrera del Aguila 1989). Access to the Andoas area is limited to contract air flights and occasional river traffic, and thus represents a unique and relatively undisturbed study site.

During the 12-mo study period, six 2-wk visits were made to the area in April, June, September, and December 1990, and February and May 1991. A total of 12 all-night human bait collections (144 h) were made. In addition, >160 h of human bait trapping were conducted both indoors and outdoors during the peak anopheline activity period in the evening (1 h before and after sunset). Anophelines were collected following standard procedures using human bait, flash-

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Table 1. Prevalence of *Plasmodium vivax* VK210 and VK247 CS proteins detected by ELISA in *Anopheles* mosquitoes collected in the Andoas Peru area

Genus (Subgenus) species	No. (%) identified	No. (% of all positive mosquitoes)			
		VK210	VK247	Both	Total
An. (Nyssorhynchus) oswaldoi complex[a]	4,354 (98.9)	10 (25.6)	28 (71.8)	2 (5.1)	38
An. (Nys.) triannulatus	9 (0.2)	0	0	0	0
An. (Ano.) mattogrossensis	40 (0.9)	1 (2.6)	0	0	1
Total	4,403	11 (28.2)	28 (71.8)	2 (5.1)	39

[a] Including *An. nuneztovari*, *An. oswaldoi*, *An. rangeli*, and *An. benarrochi*.

lights, and a hand-held aspirator (World Health Organization 1975).

Captured mosquitoes were stored overnight at room temperature (25°C) in cups wrapped with moist paper towels. The following morning, after being placed in a small freezer (0°C) for several minutes, the anopheline mosquitoes were identified using several references (Faran 1980, Faran & Linthicum 1981, Linthicum 1988). Mosquitoes were then frozen (−20°C), transported to our laboratory in Lima, and prepared for ELISA analysis.

ELISA Methods. The sporozoite infection rate was expected to be low (<1%), based on the 16% *P. vivax* prevalence in residents of the area (Franke et al. 1991); thus mosquitoes were tested in pools. Mosquitoes were divided between the meso- and metathoracic segments and the head and anterior thoracic section of five mosquitoes of the same species, and collection dates were pooled. Samples were ground in blocking buffer–0.05% Nonidet P-40 and tested in indirect ELISAs (Wirtz et al. 1991), using monoclonal antibodies NVS no. 3 and 182.1G12 developed against VK210 (Wirtz et al. 1991) and VK247 (Rosenberg et al. 1989) *P. vivax* sporozoites, respectively. Negative controls consisted of laboratory-reared *An. pseudopunctipennis* Theobald, and positive controls of a recombinant protein or a synthetic peptide containing VK210 (Wirtz et al. 1991) or VK247 (Rosenberg et al. 1989) CS protein nonapeptide repeats, respectively, were run on each microtiter plate. The lower limits of sensitivity of the VK210 and VK247 ELISAs were ≈25 (Wirtz et al. 1991) and 50 (Wirtz et al. 1992) sporozoites per well, respectively. Absorbance values (414 nm) were recorded with an ELISA plate reader (MR700, Dynatech Laboratories, Chantilly, VA) 60 min after the addition of substrate. Wells with absorbance values >3 times the mean of the five negative controls for VK247 and >2 times the mean of five negative controls for VK210, run concurrently in each plate, were selected and retested for confirmation.

Results

In total, 4,403 anophelines were captured and identified during the six visits to Andoas (Table 1). Unfortunately, a short time after all mosquitoes had been prepared for ELISA testing, we learned through collaborative work with the Walter Reed Biosystematics Laboratory, Washington, DC, of problems in the taxonomy of *Anopheles* mosquitoes in the *Nyssorhynchus* group. Enough doubt lingers with our original identifications that it would not be prudent to assume anything more than the following: >98% of the specimens that could be identified were in the subgenus *Nyssorhynchus*; almost all of those were in the Oswaldoi complex (Faran 1980). Species within this complex are particularly difficult to differentiate, and the standard taxonomic reference is presently under revision (R. Wilkerson, personal communication).

Although it is not possible to report with certainty the exact proportion of each species captured within the Oswaldoi complex, specimens confirmed by the Walter Reed Biosystematics Laboratory included *Anopheles nuneztovari* Gabaldon, *An. rangeli* Gabaldon, Cova Garcia & Lopez, *An. oswaldoi* (Peryassu), and *An. benarrochi* Gabaldon, Cova Garcia & Lopez. Nine specimens of the Triannulatus subgroup were definitively identified as *An. (Nys.) triannulatus* (Neiva & Pinto). Definitive identification was also made on 40 specimens of *An. (Ano.) mattogrossensis* Lutz & Neiva.

Because of the low *P. vivax* prevalence and CS protein rates in the region, a positive pool was assumed to contain only a single CS protein-positive mosquito. With that assumption in mind, *P. vivax* CS proteins were detected by ELISA in 0.9% (39/4,403) of the mosquitoes tested (Table 1). The VK210 CS protein was detected in 0.3% (11/4,403) of the mosquitoes, which composed 28% (11/39) of the CS protein-positive mosquitoes. The VK247 *P. vivax* CS protein was detected in 0.6% (28/4,403) of the mosquitoes, which included 72% (28/39) of the CS protein-positive mosquitoes. Mixed VK210 and VK247 infections were detected in 5.0% (2/39) of the CS protein-positive mosquitoes.

Discussion

The presence of sporozoite CS protein in mosquito samples does not incriminate a species as a malaria vector because ELISA techniques can-

not distinguish between infected and infective mosquitoes (Wirtz & Burkot 1991). When ELISA results alone are used to estimate the proportion of infective *Anopheles* mosquitoes, an overestimate is common (Beier et al. 1990; T. Klein, personal communication). Nearly 99% of the identified *Anopheles* mosquitoes captured using human bait methods in this study were in the subgenus *Nyssorhynchus* and 0.9% contained CS protein. With the exception of *An. benarrochi* and *An. mattogrossensis*, all of the species captured were considered as potential vectors because each had previously been confirmed as positive for salivary gland *P. vivax* sporozoites: *An. nuneztovari*, *An. oswaldoi*, and *An. rangeli* (Hayes et al. 1987) in Peru and *An. triannulatus* in Brazil (De Arruda et al. 1986). Ongoing vector studies in the region will soon clarify this issue. It is important to note that had we continued to rely on the use of only the VK210 ELISA in our work we would not have identified a large proportion of the sporozoite-infected mosquitoes in the region.

The high positive rate (2.5%) in the *An. mattogrossensis* mosquitoes is an interesting finding. Only 40 specimens were captured during more than 300 h of human bait trapping, and further study is required to determine the role, if any, of *An. mattogrossensis* in *P. vivax* transmission in the area.

These results indicate that future studies of potential vector(s) of *P. vivax* malaria in the Andoas region of Peru should focus on the *Nyssorhynchus* group of anophelines and that the VK247 *P. vivax* CS protein is common in the study area. In addition, it is obvious that the use of the terms "predominant" and "variant" in reference to the repeat regions of the CS protein should be eliminated.

These findings agree closely with those previously mentioned, that 60% of the *P. vivax*-positive individuals in the same area had antibodies to the variant CS protein (Franke et al. 1992). Our inability to differentiate clearly the anopheline species found in the Andoas area should not detract from the fact that both the VK210 and VK247 forms of CS protein were detected and that the rates at which the two forms of CS protein were found closely parallel the human antibody rates to the same CS proteins. These findings stress the importance of considering the VK247 epitope in constructing vaccines based on the repeat region of the *P. vivax* CS protein.

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References Cited

Beier, J. C., P. V. Perkins, J. K. Koros, F. K. Onyango, T. P. Gargan, R. A. Wirtz, D. K. Koech & C. R. Roberts. 1990. Malaria sporozoite detection by dissection and ELISA to assess infectivity of Afrotropical *Anopheles* (Diptera: Culicidae). J. Med. Entomol. 27: 377–384.

Cochrane, A. H., E. H. Nardin, M. De Arruda, M. Maracic, P. Clavijo, W. E. Collins & R. S. Nussenzweig. 1990. Widespread reactivity of human sera with a variant repeat of the circumsporozoite protein of *Plasmodium vivax*. Am. J. Trop. Med. Hyg. 43: 446–451.

De Arruda, M., M. B. Carvalho, R. S. Nussenzweig, M. Maracic, A. W. Ferreira & A. H. Cochrane. 1986. Potential vectors of malaria and their different susceptibility to *Plasmodium falciparum* and *Plasmodium vivax* in northern Brazil identified by immunoassay. Am. J. Trop. Med. Hyg. 35: 873–881.

Faran, M. E. 1980. Mosquito studies (Diptera, Culicidae) XXXIV. A revision of the Albimanus section of the subgenus *Nyssorhynchus* of *Anopheles*. Contrib. Am. Entomol. Inst. (Ann Arbor) 15(7).

Faran, M. E. & K. J. Linthicum. 1981. A handbook of the Amazonian species of *Anopheles* (*Nyssorhynchus*) (Diptera: Culicidae). Mosq. Syst. 13(1): 1–81.

Franke, E. D., C. M. Lucas & E. San Roman. 1991. Antibody response of humans to the circumsporozoite protein of *Plasmodium vivax*. Infect. Immun. 59(8): 2836–2838.

Franke, E. D., C. M. Lucas, E. San Roman & R. A. Wirtz. 1992. Prevalence of antibody to the variant repeat of the circumsporozoite protein of *Plasmodium vivax* in Peru. Am. J. Trop. Med. Hyg. 46: 708–710.

Hayes, J., G. Calderon, R. Falcon & V. Zambrano. 1987. Newly incriminated Anopheline vectors of human malaria parasites in Junin Department, Peru. J. Am. Mosq. Control Assoc. 13(3): 418–422.

Kain, K. C., J. Keystone, E. D. Franke & D. E. Lanar. 1991. Global distribution of a variant of the circumsporozoite gene of *Plasmodium vivax*. J. Infect. Dis. 164: 208–210.

Linthicum, K. J. 1988. A revision of the Argyritarsis section of the subgenus *Nyssorhynchus* of *Anopheles* (Diptera: Culicidae). Mosq. Syst. 20(2/3): 98–271.

Peñaherrera del Aguila, C. [ed.]. 1989. Atlas del Perú. Instituto Geográfico Nacional, Lima, Peru.

Rosenberg, R., R. A. Wirtz, D. E. Lanar, J. Sattabongkot, T. Hall, A. P. Waters & C. Prasittisuk. 1989. Circumsporozoite protein heterogeneity in the human malaria parasite *Plasmodium vivax*. Science (Washington, DC) 245: 973–976.

Wirtz, R. A. & T. R. Burkot. 1991. Detection of malarial parasites in mosquitoes, pp. 77–106. *In* K. F. Harris [ed.], Advances in disease vector research. Springer-Verlag, New York.

Wirtz, R. A., R. Rosenberg, J. Sattabongkot & H. K. Webster. 1990. Prevalence of antibody to heterologous circumsporozoite protein of *Plasmodium vivax* in Thailand. Lancet 336: 593–595.

Wirtz, R. A., J. Sattabongkot, T. Hall, T. R. Burkot & R. Rosenberg. 1992. Development and evaluation of an enzyme-linked immunosorbent assay for *Plasmodium vivax*-VK247 sporozoites. J. Med. Entomol. 29: 854–857.

Wirtz, R. A., Y. Charoenvit, T. R. Burkot, K. M. Esser, R. L. Beaudoin, W. E. Collins & R. G. Andre. 1991. Evaluation of monoclonal antibodies against *Plasmodium vivax* sporozoites for ELISA development. Med. Vet. Entomol. 5: 17–22.

World Health Organization. 1975. Manual on practical entomology in malaria. Part II. Methods and techniques. WHO Offset Publ. 13.

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